MOLECULAR AND CELLULAR BIOLOGY, July 1997, p. 3589–3599 Vol. 17, No. 7
0270-7306/97/$04.00+0

Sequence-Specific Epigenetic Effects of the Maternal Somatic Genome on Developmental Rearrangements of the Zygotic Genome in *Paramecium primaurelia*

ERIC MEYER,* ALAIN BUTLER, KARINE DUBRANA, SANDRA DUHARCOURT, AND FRANÇOIS CARON

Laboratoire de Génétique Moléculaire, ENS, 75005 Paris, France

Received 6 March 1996/Returned for modification 25 April 1996/Accepted 22 April 1997

In ciliates, the germ line genome is extensively rearranged during the development of the somatic macronucleus from a mitotic product of the zygotic nucleus. Germ line chromosomes are fragmented in specific regions, and a large number of internal sequence elements are eliminated. It was previously shown that transformation of the vegetative macronucleus of *Paramecium primaurelia* with a plasmid containing a subtelomeric surface antigen gene can affect the processing of the homologous germ line genomic region during development of a new macronucleus in sexual progeny of transformed clones. The gene and telomere-proximal flanking sequences are deleted from the new macronuclear genome, although the germ line genome remains wild type. Here we show that plasmids containing nonoverlapping segments of the same genomic region are able to induce similar terminal deletions; the locations of deletion end points depend on the particular sequence used. Transformation of the maternal macronucleus with a sequence internal to a macronuclear chromosome also causes the occurrence of internal deletions between short direct repeats composed of alternating thymines and adenines. The epigenetic influence of maternal macronuclear sequences on developmental rearrangements of the zygotic genome thus appears to be both sequence specific and general, suggesting that this *trans*-nucleus effect is mediated by pairing of homologous sequences.

Nuclear dimorphism is a characteristic feature of all ciliates. It depends on the developmental process of nuclear differentiation, which offers a unique opportunity for researchers to study programmed DNA rearrangements in eukaryotes. Each vegetative cell contains two types of nuclei with different structures and functions. The micronuclei are small, diploid nuclei that remain transcriptionally silent through most of the life cycle. They are often called germ line nuclei because their main function is to produce haploid gametic nuclei during meiosis and transmit genetic information to the following sexual generation. In contrast, macronuclei are large, highly polyploid nuclei in which all vegetative transcription takes place. Macronuclei divide amitotically and have a lifetime restricted to the vegetative part of the life cycle; during sexual processes, they stop replicating and eventually disappear. After fertilization, new macronuclei differentiate from mitotic products of the diploid zygotic nucleus while other products become the new micronuclei. Thus, in a vegetative cell, all nuclei derive from the micronuclei of the previous sexual generation (for a complete description of these processes in *Paramecium aurelia*, see reference 43).

During development of the macronucleus from a diploid nucleus, the germ line genome is amplified to a high ploidy level (~1,000*n* in *P. aurelia* species) and extensively rearranged at specific sites (for reviews, see references 9, 23, and 34). Chromosomes are fragmented into shorter, acentromeric molecules healed by the addition of telomeric repeats, and numerous internal sequences are eliminated from coding and noncoding sequences. *Paramecium* internal eliminated sequences (IESs) are short, single-copy, noncoding elements that are invariably excised between 5′-TA-3′ direct repeats, one of which is left in the rearranged macronuclear sequence. Some of these rearrangements are very precise, always occurring at the same nucleotide of the germ line sequence. This precision is needed for the removal of IESs interrupting coding sequences (40, 44). In some cases, however, the exact boundaries of the deleted segments may vary by a few base pairs (13, 38, 45). In *Paramecium* species, addition of telomeric hexanucleotide repeats after fragmentation of germ line chromosomes is not precise at the nucleotide level but rather occurs at seemingly random positions within broadly defined, but reproducible, telomere addition regions (3, 16). The sizes of these telomere addition regions vary from 200 bp to more than 1 kb for different fragmentation sites or between different caryonidal clones (vegetative clones arising from single events of macronuclear development). Alternative rearrangements of a single germ line sequence have also been described for several species. They can be distinguished from imprecise rearrangements in that they yield several discrete classes of rearranged molecules. Each of these classes may show some sequence microheterogeneity. Fragmentation of germ line chromosomes in *Paramecium* species, for example, often produces several classes of macronuclear molecules, corresponding to the use of alternative telomere addition regions spaced by 2 to 13 kb (1, 5, 16, 39).

Because the macronucleus differentiates from the micronuclear lineage, it is generally assumed that the rearrangement program is entirely encoded in the germ line genome, i.e., that the rearrangement patterns are solely determined by *cis*-acting sequence elements directing the action of developmentally regulated enzymes. However, the study of entirely homozygous strains of *Paramecium* species has revealed the important role played by the old macronucleus (the prezygotic maternal macronucleus) in specifying rearrangement patterns in the developing macronucleus (for reviews, see references 30 and 31). Variant rearrangement patterns can be maternally inherited in

* Corresponding author. Mailing address: Laboratoire de Génétique Moléculaire, ENS, 46 Rue d'Ulm, 75005 Paris, France. Phone: 33 (1) 44 32 39 49. Fax: 33 (1) 44 32 39 41. E-mail: emeyer@wotan.ens.fr.

cell lines with strictly identical germ line genomes. In wild-type strain 51 of *Paramecium tetraurelia*, fragmentation of the germ line chromosome bearing the *A* surface antigen gene results in the formation of macronuclear telomeres in three alternative regions located 8, 13, and 26 kb downstream of the gene. In the d48 variant strain, the macronuclear genome shows a terminal deletion of the *A* gene; during macronuclear development, telomeres are added in a single region located at the 5′ end of the gene (16). This variant rearrangement pattern is not inherited as a Mendelian trait; genetic crosses and nuclear transplantation experiments have shown that the d48 germ line genome is entirely wild type (24). Which rearrangement takes place in the developing macronucleus is determined by the old macronucleus through unknown *trans*-acting factors that can be detected in the cytoplasm at the time of nuclear reorganization (25). A similar maternal inheritance has also been observed for variant rearrangement patterns in the *B* gene region (41) and for the excision of some IESs, which can be excised in the developing macronucleus only if they are absent from the old macronuclear genome (13a, 14).

In a previous paper, we reported experiments showing that the processing of the germ line *G* gene of *Paramecium primaurelia* is sensitive to the structure and copy number of homologous sequences in the old macronucleus (29). Transformation of the vegetative macronucleus by microinjection of a high copy number of a plasmid containing the *G* gene results in a different rearrangement pattern in the sexual progeny of transformed clones. The *G* gene is deleted from the new macronuclear genome; the macronuclear telomere normally positioned about 5 kb downstream from the *G* gene now forms upstream of the coding sequence. This terminal deletion shows some heritability through sexual reproduction, as processing of the germ line *G* gene region is also affected in the following generation, resulting in a wide variety of different telomere positions. After several cycles of nuclear reorganization, a new cell line was established which, like the d48 strain of *P. tetraurelia*, reproducibly uses a variant telomere addition region. Here again, genetic analyses confirmed that the germ line genome remains wild type.

Both the maternal inheritance of variant rearrangements and the effects of transformation of the old macronucleus on the rearrangements occurring in the developing macronucleus indicate that some sequence-specific information is transmitted between these two nuclei. This phenomenon has so far been observed only with genes coding for surface antigens, such as the *A*, *B*, and *G* genes, all of which are located near macronuclear telomeres. In the present work, we have addressed the question of the generality of the phenomenon by transforming the vegetative macronucleus with plasmids containing different fragments of the macronuclear genome. All five plasmids tested induced deletions of the homologous region in the developing macronucleus at the following autogamy. Different fragments of the *G* gene region induced different terminal deletions; a fragment internal to a macronuclear chromosome induced internal deletions only.

MATERIALS AND METHODS

Cell lines and cultivation. *P. primaurelia* wild-type strain 156 (43) is a well-characterized stock strain that has been used extensively in genetic studies of the surface antigen system. Cells were grown in a grass infusion medium bacterized with *Klebsiella pneumoniae* the day before use and supplemented with 0.8 mg of β-sitosterol (Merck, Darmstadt, Germany) per ml. Cultivation and autogamy were carried out at 24°C. Basic methods of cell culture utilized have been described previously (42).

Microinjections. Caryonidal clones that had undergone 15 to 30 fissions were injected in Dryl's buffer (12) containing 0.2% bovine serum albumin, under an oil film, while being visualized with a phase-contrast inverted microscope (Axiovert 35M; Zeiss). Approximately 5 pl of a solution of CsCl-purified plasmid DNA, which had been filtered with a 0.22-μm-pore-size Millex-GV4 filter (Millipore) and dissolved in water at a concentration of 5 mg/ml, was delivered into the macronucleus.

Autogamy. Autogamy was induced by starving the cells after they had reached the appropriate clonal age and assessed by staining with a 15:1 (vol/vol) mixture of carmine red (0.5% in 45% acetic acid) and fast green (1% in ethanol). Cells were isolated from depressions showing more than 50% autogamous cells; autogamy was checked by staining three out of four cells after the second division of the isolated cell to verify the presence of macronuclear fragments.

Dot blot analyses. About 500 cells were pipetted from depression slide cultures and transferred to 400 μl of 0.4 N NaOH–50 mM EDTA. The lysates were incubated for 30 min at 68°C and loaded on a Hybond N^+ membrane (Amersham, Little Chalfont, United Kingdom) by using a dot blot apparatus. The membrane was kept wet with 0.4 N NaOH for 15 min, washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and subsequently treated as a Southern blot.

DNA extraction. Cultures of exponentially growing cells (400 ml at 1,000 cells/ml) were centrifuged. After being washed in Dryl's buffer (12), the pellet was resuspended in 1 volume of the same buffer and quickly added to 4 volumes of lysis solution (0.44 M EDTA [pH 9.0], 1% sodium dodecyl sulfate [SDS], 0.5% *N*-lauroylsarcosine [Sigma], and 1 mg of proteinase K [Merck] per ml) at 55°C. The lysate was incubated at 55°C for at least 5 h, gently extracted once with phenol, and dialyzed twice against TE (10 mM Tris-HCl–1 mM EDTA, pH 8.0) containing 20% ethanol and once against TE.

DNA restriction and electrophoresis. DNA restriction and electrophoresis were carried out according to standard procedures (37). Pulsed-field electrophoresis was carried out in a homemade contour-clamped homogeneous electric field (CHEF) apparatus (7) in 0.25× TBE (1× TBE is 89 mM Tris, 89 mM borate, and 2.5 mM EDTA) at 5 V/cm, with cooling to 8°C and a commutation period of 4 s.

Southern blotting. DNA was transferred from agarose gels to Hybond N^+ membranes (Amersham) in 0.4 N NaOH after depurination in 0.25 N HCl. Hybridization was carried out according to the procedure described in reference 8 in 7% SDS–0.5 M sodium phosphate–1% bovine serum albumin–1 mM EDTA (pH 7.2) at 63°C. Probes were labeled to a specific activity of 3.10^9 cpm/μg by using a random priming kit (Boehringer, Mannheim, Germany). Membranes were then washed for 30 min in 0.2× SSC–0.5% SDS at 60°C prior to autoradiography.

PCR amplification of telomeric fragments from terminal deletions. *G* gene telomeric fragments were amplified with primer 1 (5′-GATTCTTCAGAAATC AAAGAAATACTTAATT-3′, matching a sequence 15 bp upstream of the *Bam*HI site) and primer 2, a degenerate oligonucleotide designed to match all possible combinations of the two types of *Paramecium* telomeric repeats [5′-((C /A)AACCC)$_5$-3′], in a Perkin-Elmer Cetus thermocycler. Reactions were carried out in capped 0.5-ml Sigma polypropylene tubes with reaction mixtures (25 μl) containing 40 to 800 ng of total DNA, 1× PCR buffer (supplied by the manufacturer [Promega]), 80 μM each deoxynucleoside triphosphate, 2 μM each oligonucleotide, and 0.8 U of *Tfl* polymerase. Amplifications were run for 20 cycles (92°C, 1 min; 65°C, 1 min 15 s; 74°C, 1 min).

PCR amplification and sequencing of internal deletion junctions. Deletion junctions were amplified with primer 3 (5′-CTAATACAGTCAATCCTGAAT ACTTTAGTCATG-3′; the 5′ end matches position 72 of the wild-type sequence) and either primer 4 (5′-CCATTGAAATTTGTGAAGTCACCTCCTT GAGCC-3′; the 5′ end matches position 6388; used for deletions 4 and 5) or primer 5 (5′-ATCCATTAATGCTATCCAATTTTCCATATTCTC-3′; the 5′ end matches position 7494; used for all other deletions) from total DNA of clones K1 (deletions 5 to 7 and 10 to 12) or K2 (deletions 1 and 2) or from size-selected *Hinc*II-*Eco*NI fragments from clone K2 (3.3 to 3.6 kb for deletions 5, 8, and 9; 4.8 to 5.1 kb for deletions 3 and 4). PCR reactions were run for 30 cycles under the same conditions as for telomeric amplifications, except that the annealing temperature was 63°C and the extension time was increased to 2 min. PCR products were cloned into plasmid pCRScript (Stratagene) either in bulk or after size selection, and clones containing inserts of various sizes were sequenced with the Sequenase sequencing kit (U.S. Biochemical Corp.) until a deletion junction could be identified by comparison with the wild-type sequence. In most cases, the size of the insert was in good agreement with the deduced length of the deleted segment and the insert was not sequenced further; a few clones resulting from nonspecific priming of one oligonucleotide at a different position of the sequence were discarded.

Sequence analysis. The sequence was analyzed with the programs Blastx, Gap, Window, and Statplot from the Wisconsin Package version 9.0 (Genetics Computer Group, Madison, Wis.).

Nucleotide sequence accession number. The nucleotide sequence of the 8,033-bp segment of the wild-type macronucleus has been submitted to the EMBL database and given accession no. Y13117.

RESULTS

Macronuclear deletions can be induced by different sequences. The 9.0-kb macronuclear DNA fragment cloned into

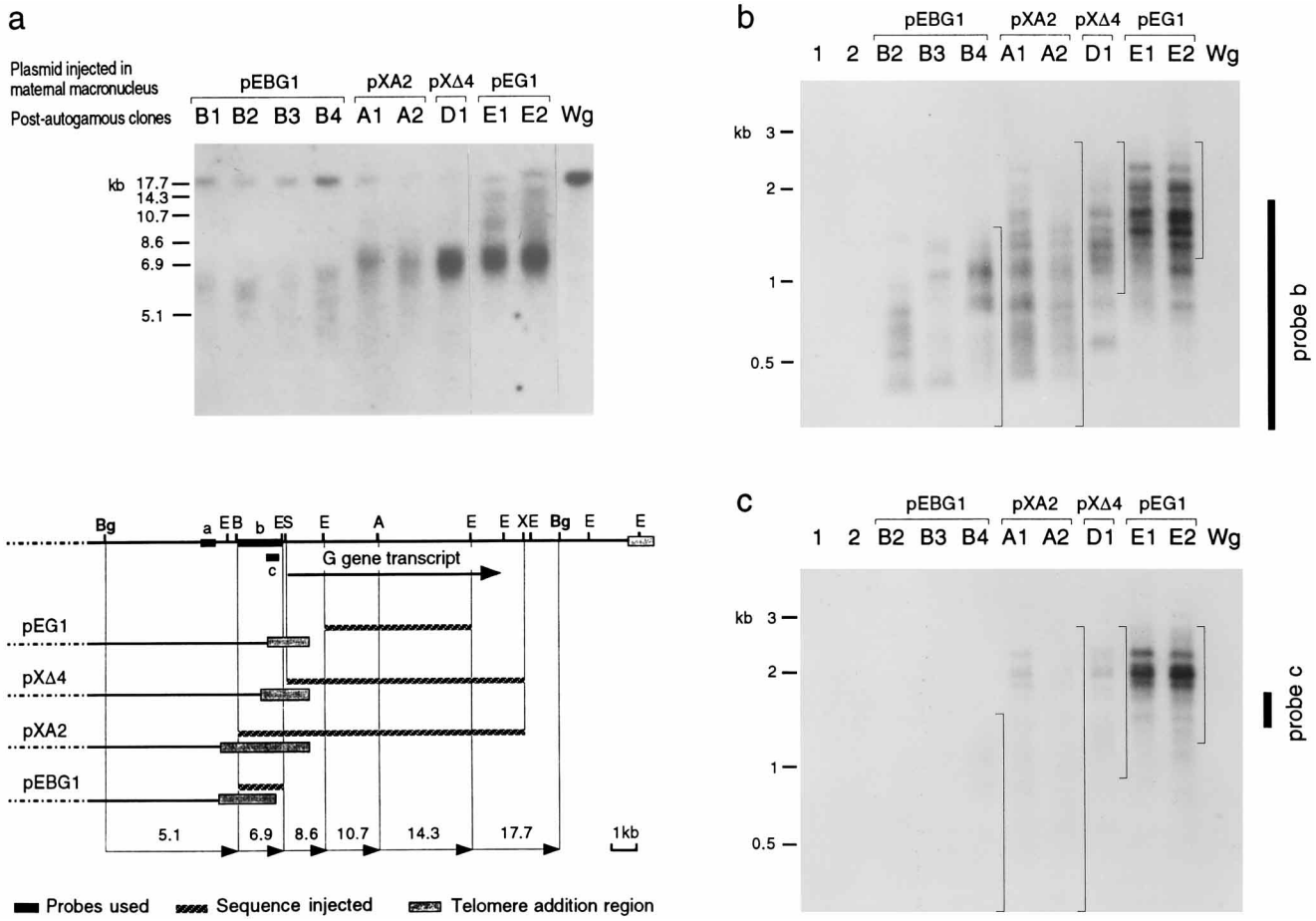

FIG. 1. Plasmid-induced terminal deletions in the *G* gene region. (a) Southern blot of *Bgl*II-restricted genomic DNA from postautogamous caryonidal clones derived from clones transformed with plasmids pEBG1 (lanes B1 to B4), pXA2 (lanes A1 and A2), pXΔ4 (lane D1), and pEG1 (lanes E1 and E2) or from an uninjected control (lane Wg). The probe used is a 400-bp *Xba*I-*Eco*RI fragment located 3.5 to 3.9 kb downstream of the left *Bgl*II site. The line drawing shows the positions of plasmid inserts (hatched bars) and induced telomere addition regions (shaded boxes) relative to the restriction map of the wild-type macronuclear region (top line). Restriction sites: A, *Asp*718; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; X, *Xba*I. (b) Southern blot of PCR-amplified telomeric fragments from the same clones. Lanes 1 and 2, PCR control amplifications of DNA from clone B2 with each primer alone (primer 1 is located just upstream of the *Bam*HI site; primer 2 is the telomeric oligonucleotide). The brackets on the right side of each series of clones show the expected size range of amplified fragments, based on the genomic Southern blot mapping. The black bar on the right side shows the extension of sequences covered by probe b (fragment EBG1; see map). (c) Same blot as in panel b, after stripping and rehybridization with probe c, a 423-bp *Dra*I-*Xba*I subfragment of EBG1 (see map). The traces of signal below the indicated position of probe c are due to incomplete removal of probe b. The black bar on the right shows the extension of sequences covered by probe c.

the previously studied pXΔ4 plasmid contains the transcribed sequence of the *G* gene, as well as 230 bp upstream and 510 bp downstream of the mature transcript. Three additional plasmids containing different fragments of the *G* gene region (see Fig. 1) were microinjected into the macronuclei of vegetative cells of strain 156 to test whether new telomere positions could be induced after nuclear reorganization. Plasmid pEG1 contains a 5.7-kb fragment internal to the coding sequence and thus lacks the promoter region that presumably allows transcription of the *G* gene from the pXΔ4 plasmid. Plasmid pEBG1 contains a 1.9-kb fragment located upstream of the *G* gene (orientation is defined according to the direction of *G* gene transcription). Plasmid pXA2 contains a 10.9-kb fragment which includes the fragments cloned into pEBG1 and pXΔ4 as well as the 50-bp stretch lying between them. Plasmids were injected as supercoiled circular molecules at a high concentration. After injection, circular molecules are linearized at many different points of the sequence (18). Telomeric repeats are added to the extremities of linear monomers or multimers, which then replicate autonomously in the macronucleus (17, 20, 29). Injected cells were grown in depression slides, and the amount of replicating plasmid was determined by a quick dot blot procedure (see Materials and Methods). Selected cell lines maintaining between 10,000 and 80,000 plasmid copies per macronucleus (i.e., between 10 and 80 times the normal amount of the gene) were grown for DNA extraction. Plasmid copy numbers were more precisely measured by scanning autoradiographs of Southern blots and comparing the plasmid signal with the endogenous *G* gene signal. Transformed lines were then allowed to undergo autogamy, a self-fertilization sexual process resulting in entirely homozygous progeny (43). Isolated caryonides were grown, and their DNA was extracted to probe the structure of the new macronuclear genome. Figure 1a shows a Southern blot of *Bgl*II-restricted genomic DNA which was probed with a fragment located upstream of the *G* gene (probe a). This probe reveals a 17.7-kb restriction fragment in wild-type DNA (lane Wg). The previously studied pXΔ4-induced deletion (29) yields a telomeric fragment showing up as a 2-kb smear centered around 7.0 kb (lane D1). Similarly, postautogamous caryonides derived from cell lines

transformed with the other three plasmids show a greatly reduced density of the wild-type band at 17.7 kb; instead, they show the broad smears characteristic of telomeric fragments. The putative new telomere addition regions can be mapped by estimating the size range of these fragments (see the schematic drawing in Fig. 1). A second mapping, using *Bam*HI restriction and fragment EBG1 as a probe, gave compatible results (data not shown).

To confirm the terminal nature of the deletions, a PCR amplification was performed with one oligonucleotide located upstream of the *Bam*HI site (primer 1) and a second, degenerate oligonucleotide complementary to *Paramecium* telomeric repeats (primer 2) (Fig. 1b). In the control caryonide (lane Wg), the absence of any specific amplification product indicates that no telomeres are added within PCR reach of primer 1 in the wild type. In all postautogamous caryonides derived from transformed cells, DNA molecules heterogeneous in size can be amplified and specifically revealed with fragment EBG1 (probe b [Fig. 1b]). Although the PCR amplification is expected to be biased in favor of the shortest molecules, the size range of these fragments agrees well with the mapping of new telomere addition regions. The absence of signal below ~0.3 kb likely reflects poor transfer or hybridization of very short fragments. Thus, all four plasmids, when injected into the old macronucleus, induce terminal deletions of the homologous germ line genomic region in the developing macronucleus, even though some of them bear no common sequence.

Different sequences induce different terminal deletions. Plasmid pEG1, a subclone of pXΔ4, induces terminal deletions that map essentially to the same region as the pXΔ4-induced deletions. In Fig. 1a (lanes E1 and E2), traces of hybridizing material can be seen between the bulk of the telomeric distribution and the wild-type band, but it is not clear whether this material is a specific consequence of pEG1 transformation, because it is also present in some wild-type controls (see Fig. 2, lanes W1 and W2). In contrast, plasmid pEBG1 induces telomere formation in a region that is significantly displaced upstream (Fig. 1a, lanes B1 to B4). The weakness of the signal is due to the larger smear of short telomeric fragments in the agarose gel. It is also possible that telomere formation occurs upstream of the probe in a fraction of macronuclear molecules, resulting in a decreased number of copies of the probe a sequence in the new macronucleus. The Southern blot of PCR products (Fig. 1b) also shows clear differences in the average sizes of amplified telomeric fragments. These differences are further highlighted by rehybridization of the same blot with a 0.4-kb subfragment of EBG1 located close to the *Eco*RI site (probe c [Fig. 1c]); fragments amplified from pEBG1-induced deletions are shorter and, in contrast to those amplified from pEG1-induced deletions, do not extend sufficiently downstream to be efficiently revealed by probe c. Finally, it should be noted that plasmid pXA2, which contains both the pEBG1 and the pEG1 sequences, induces telomere formation in an extended region overlapping both mutant telomere addition regions. Thus, the injection of different fragments leads to the formation of telomeres in different regions during the subsequent nuclear reorganization. The mutant telomere addition region always begins upstream of the injected sequence, partially overlapping it in the case of pEBG1, pXA2, and pXΔ4; the pEG1-induced telomeric distribution, however, mainly lies upstream of the EG1 sequence.

Effect of transformation of mutant macronuclei. It has been reported that the similar macronuclear mutant d48 of *P. tetraurelia* could be rescued by injection into the macronucleus of some fragments of the coding sequence that are missing in the mutant macronuclear genome. After autogamy of transformed cell lines, it was observed that the number of molecules containing a complete *A* gene in the new macronucleus was increased compared to uninjected controls, implying that the injection of these fragments stimulates the formation of telomeres downstream of the coding sequence (19, 21, 25, 47, 48). Although plasmid-induced *G* gene deletions are less stable than the d48 deletion, showing partial spontaneous reversion at the following autogamy (29), we asked whether microinjection of deleted sequences into a first-generation mutant would enhance *G* gene amplification after a second autogamy. One of the mutants derived from a pEBG1-transformed cell (clone B1 in Fig. 1 and 2) was microinjected with plasmids pEBG1 (55,000 copies per macronucleus) or pEG1 (about 5,000 copies). After autogamy of transformed lines, individual caryonides were grown and their macronuclear genomes were analyzed as described above (except that a different probe was used to achieve better labelling of the mutant telomeric fragments) and compared to control caryonides obtained by autogamy of the uninjected B1 mutant (lanes 17 to 21 in Fig. 2). The latter show the high-level variability of telomere position previously observed after autogamy of first-generation pXΔ4-induced mutants (29). Two of them (lanes 19 and 21) retain telomeres in the B1 mutant telomere addition region, although they appear to be a subset of the sites used in the first-generation mutant. One (lane 17) primarily shows the wild-type band. The other two (lanes 18 and 20) appear to have telomeres at multiple intermediate positions. Lanes 1 through 4 show four caryonides derived from the pEBG1-transformed B1 mutant; these samples clearly contain less of the wild-type telomere addition region than control caryonides from the uninjected B1 mutant. Lanes 5 through 16 show 12 caryonides derived from the pEG1-transformed B1 mutant. The density of the wild-type band differs in these caryonides, but on average it is not higher than that seen in the control caryonides (lanes 17 to 21). Thus, in contrast to the d48 rescue experiments, injection of these plasmids into the mutant clone did not enhance reversion to the wild-type telomere addition region.

Figure 2 also includes, for comparison, the postautogamous caryonides derived from transformed wild-type cells (lanes B1 to B4, E1, and E2; as in Fig. 1). The comparison of lanes 1 to 4 with lanes B1 to B4 and lanes 5 to 16 with lanes E1 and E2 shows that for each plasmid the same telomeric distribution was induced in the new macronucleus whether the plasmid was injected into B1 or wild-type macronuclei (although the pEBG1-induced distribution is slightly displaced upstream in the case of the mutant line). A comparatively denser wild-type band is seen in the postautogamous caryonides derived from the pEG1-transformed B1 mutant (lanes 5 to 16) than in those derived from the pEG1-transformed wild-type cells (lanes E1 and E2). This is probably due to the relatively low copy number (5,000 per macronucleus) of pEG1 in the transformed mutant lines and not to a behavior difference between mutant and wild-type cells. Indeed, the ratio of deleted molecules to wild-type molecules in the new macronucleus correlates with the copy number of the injected plasmid (29).

Induction of internal macronuclear deletions. To determine whether the induction of deletions by plasmid transformation is limited to genomic regions that are close to a macronuclear telomere or to surface antigen genes, the experiment was repeated with an arbitrarily chosen internal sequence. Plasmid pLK1, which contains a 3.6-kb *Eco*RI fragment located 72 kb upstream of the *G* gene telomere (Fig. 3), was microinjected into the macronuclei of wild-type cells. A high mortality rate was observed after autogamy of transformed clones. Two surviving postautogamous caryonides derived from a clone main-

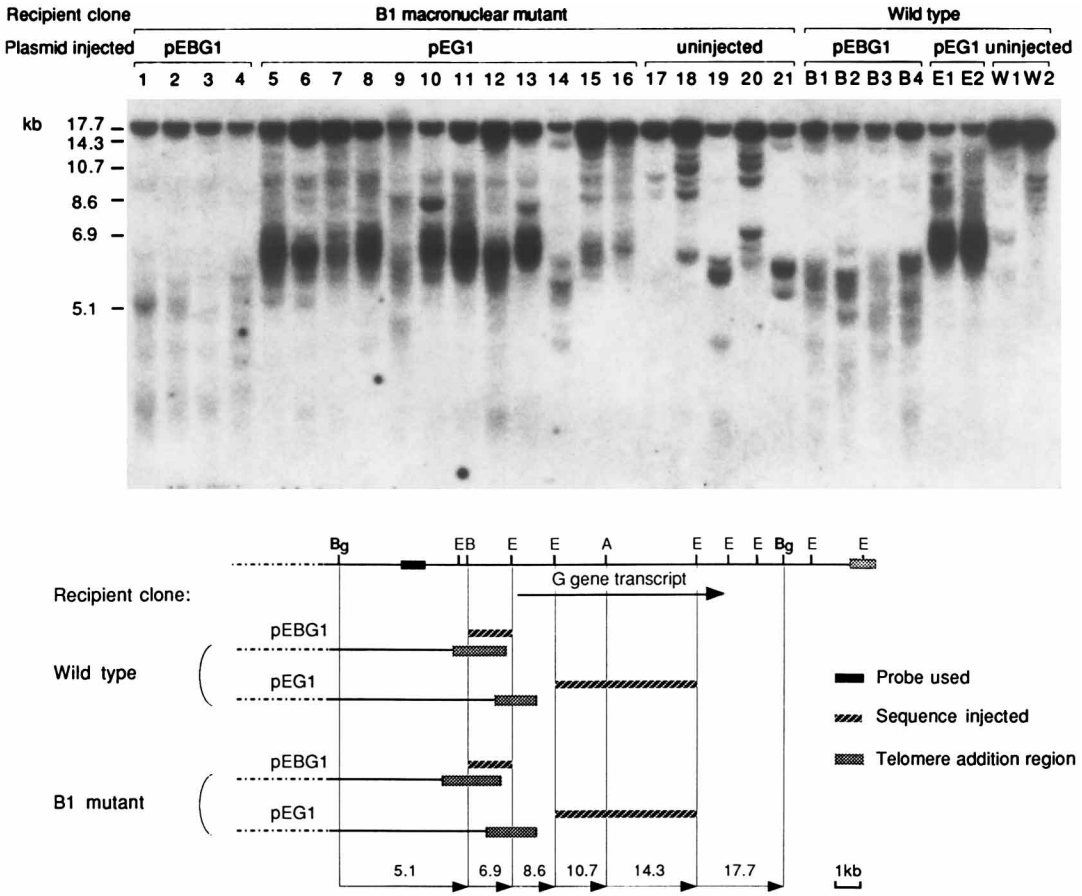

FIG. 2. Terminal deletions in postautogamous progeny of the B1 macronuclear mutant (lane B1 in Fig. 1 and this figure) transformed with plasmid pEBG1 or pEG1. The blot shows *Bgl*II-restricted genomic DNA hybridized with a 700-bp *Hin*dIII fragment located 2.6 to 3.3 kb downstream of the left *Bgl*II site. Lanes: 1 to 4, postautogamous caryonides derived from the pEBG1-transformed B1 mutant; 5 to 16, postautogamous caryonides derived from the pEG1-transformed B1 mutant; 17 to 21, postautogamous caryonides derived from the uninjected B1 mutant; B1 to B4 and E1 to E2, the same samples as in Fig. 1 (postautogamous caryonides derived from wild-type cells transformed with pEBG1 and pEG1, respectively); W1 and W2, wild-type controls. Restriction site symbols and line drawings are as described in the legend to Fig. 1.

taining about 24,000 copies of pLK1 per macronucleus were grown, and their macronuclear genomes were analyzed by probing an *Eco*RI Southern blot with the LK1 sequence. These two clones still showed the 3.6-kb fragment; however, the signal was less intense than in uninjected controls (data not shown). Taking advantage of the presence of an *Asp*718 site within the LK1 sequence, we were able to show by CHEF electrophoresis that a significant fraction of the new macronuclear chromosomes had lost this site. Figure 3 shows a Southern blot of *Asp*718-restricted genomic DNA from these two clones (lanes K1 and K2), as well as postautogamous caryonides derived from an uninjected control (lane Wk) and from clones transformed with 24,000 or 48,000 copies (lanes C1 and C2, respectively) of a control yeast plasmid, YEpJB1-23-0 (29). In addition to the site in LK1, *Asp*718 cleaves the *G* gene chromosomes at sites 105 kb upstream and 65 kb downstream of LK1. As shown in Fig. 3, the 105- and 65-kb fragments are revealed with probes a and b, respectively. Several additional fragments of lower molecular weight are also detected with probe a, due to cross-hybridization with a different macronuclear chromosome family (5). In caryonidal clones K1 and K2, a 170-kb fragment, which can be interpreted as resulting from an internal deletion of the LK1 *Asp*718 site, is detected with both probes. A very weak signal at 170 kb is also observed with probe a in controls Wk, C1, and C2; this probably also represents cross-hybridization with a different genomic region, since no such signal is seen with probe b. No chromosome breakage in or around the LK1 sequence, which would produce macronuclear chromosomes corresponding to the right or left part of the chromosome, could be detected on a Southern blot of uncut DNA which had been subjected to CHEF electrophoresis (data not shown).

Judging from molecular weight estimates, the deletion should be smaller than 10 kb. Different restriction sites flanking the LK1 sequence were then used to estimate the size of the deletion, which is entirely contained within a ~12-kb *Hin*cII fragment in the wild-type sequence (data not shown). The restriction pattern observed in deleted clones reveals a set of multiple but discrete deletions, in contrast to the terminal deletions. These discrete deletions appear to have identical sizes in clones K1 and K2, although the ratio of deleted to wild-type molecules differs between them. In both mutant caryonides, the sizes of the most abundant deleted fragments imply deletions of about 3.4 and 5.0 kb, respectively. These deletions were then mapped on Southern blots by using multiple digests and different probes. Digestion of genomic DNA with *Hin*cII and *Eco*NI yields an 8.2-kb wild-type fragment and two major deleted fragments of 4.8 and 3.2 kb (data not shown). Figure 4

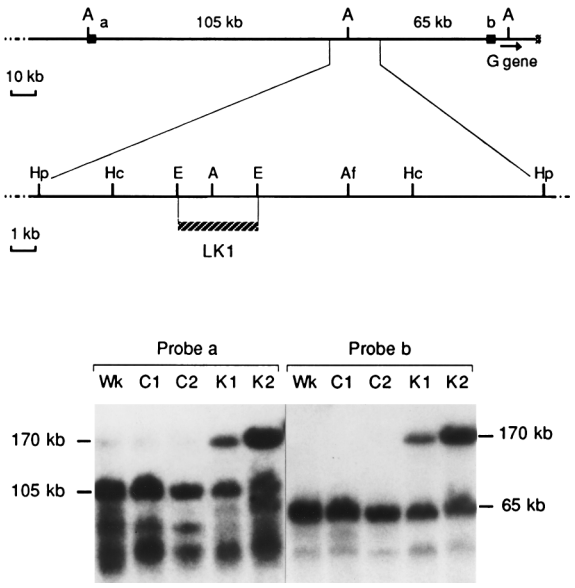

FIG. 3. Internal deletions of the LK1 sequence in postautogamous caryonides derived from pLK1-transformed wild-type cells. The line drawing shows a large-scale map of the macronuclear chromosome and a smaller-scale map of the region containing the injected LK1 sequence. The bottom panel shows a Southern blot of a 1% agarose CHEF electrophoresis gel of *Asp*718-restricted genomic DNA, hybridized successively with probes a and b (see the map for the locations of the probes). The presence of the 170-kb fragment indicates that the *Asp*718 site is absent from a large number of macronuclear copies in clones K1 and K2 (postautogamous caryonides derived from a pLK1-transformed clone) but not in Wk (wild-type control) or in C1 and C2 (postautogamous caryonides derived from clones transformed with a different plasmid). Restriction sites: A, *Asp*718; Af, *Afl*III; E, *Eco*RI; Hc, *Hin*cII; Hp, *Hpa*II.

(probe c) shows that although *Acc*I recuts the *Hin*cII-*Eco*NI wild-type fragment to 6.2 kb, it does not affect the deleted fragments, indicating that the deletions include the *Acc*I site. Similarly, *Hae*III recuts only the wild-type fragment, and therefore this site is absent from both deleted forms, whereas *Bam*HI is retained in the 4.8-kb deleted form but not in the 3.2-kb deleted form. The *Ava*II site immediately to the left of probe c is present in both deleted forms. Hybridization of the same blot with probe d (see the map in Fig. 4) shows that the 4.8-kb deleted form retains part of this sequence, indicating that the downstream end point of this deletion must lie between the *Hae*III and the *Eco*RI sites. Thus, the right deletion end points have been mapped with precisions of 0.2 kb for the 3.4-kb deletion and 0.8 kb for the 5.0-kb deletion. The left end points are located upstream of the *Acc*I site; the uncertainty about their exact positions is higher than for the right end points because it also depends on estimated deletion sizes.

Sequence analysis of pLK1-induced deletions. To characterize more precisely the deletion end points and junctions, the wild-type macronuclear sequence was determined over an 8,033-bp segment including sequence LK1. A database similarity search identified three regions (a, b, and c [Fig. 5]) potentially encoding polypeptides with very significant similarities to known proteins (cAMP-dependent protein kinases, peptidylprolyl isomerases, and cytidylate/adenylate kinases, respectively). In addition, sequence LK1 contains a 2-kb-long open reading frame (ORF in Fig. 5). The presence of these genes could explain the mortality observed after autogamy of pLK1-transformed clones. If some of them are essential, only those clones with a sufficient copy number of wild-type molecules are expected to survive. Thus, the relatively low proportion of deleted molecules in the new macronucleus, compared to terminal deletions induced by *G* gene plasmids, does not necessarily reflect a lower efficiency of induction of internal deletions.

Primers matching sequences on either side of the deletions were used in PCR amplifications of total genomic DNA from control and mutant clones (see the map in Fig. 4). Under the PCR conditions used (see Materials and Methods), no amplification product could be detected with control clone Wk, presumably because the 6.1- and 7.2-kb fragments expected from the wild-type sequence with primer pairs 3-4 and 3-5, respectively, are too large. With clones K1 and K2, however, multiple shorter fragments were readily amplified. After cloning into a plasmid, inserts of various sizes were sequenced. Figure 5 shows the sequences of 12 different deletion junctions. The sample is intended to give an overview of the structure and variability of junctions, but because of the PCR size bias it is not expected to be representative of the relative abundance of each type of deletion in the macronucleus. Indeed, junctions resulting from the smallest deletions, which correspond to the largest molecules, could be amplified only after size selection of *Hin*cII-*Eco*NI genomic fragments. Four of the junctions were obtained several times; junction 5 was sequenced from both the K1 and K2 mutant clones.

As expected from the discrete banding pattern of deleted macronuclear molecules (Fig. 4), deletion boundaries appear to be clustered at a few recombination hot spots (see the schematic drawing of deletions in Fig. 5). The 12 left boundaries fall within three short (≤16-bp) stretches of A- and T-rich DNA, and the 12 right boundaries fall within four such stretches. The locations of these hot spots are in good agreement with the restriction mapping. Deletions 3 and 4, which have their right boundaries in between the *Hae*III and *Eco*RI sites at the right end of sequence LK1, are likely to represent the smallest of the two major deletions mapped in Fig. 4, as they were amplified from size-selected *Eco*NI-*Hin*cII genomic fragments in the 4.8- to 5.1-kb range. The discrepancy between the estimated size of the deletion (~3.4 kb) and the actual sizes of deletions 3 and 4 (4.1 kb) may arise from imprecision in the mapping of the *Hin*cII site, which was based on size estimates of large fragments. Similarly, deletions 7 through 10 are likely to represent the largest of the mapped deletions, since two of them were amplified from size-selected *Eco*NI-*Hin*cII genomic fragments in the 3.3- to 3.6-kb range. Here also, actual deletion sizes (5.5 kb) are larger than the estimated 5.0 kb. Some of the minor deleted fragments visible on the Southern blot shown in Fig. 4 may correspond to other sequenced junctions or to different combinations of identified left and right hot spots (three of them are used in conjunction with more than one other in the sample of sequenced junctions).

Alignment of the sequences of junctions 2 to 12 with the wild-type sequence suggests that deletions occur between two direct repeats, one of which remains in the rearranged sequence (Fig. 6). The direct repeats are 3 to 12 bp in length and are almost exclusively composed of strictly alternating thymines and adenines. Junction 1 is peculiar in that there are no direct repeats at the deletion boundaries; moreover, the junction contains eight successive 5′-TA-3′ dinucleotides, only seven of which can be accounted for by the wild-type macronuclear sequence (Fig. 6). The extra TA might result from one deletion boundary being located within an IES sequence that is normally excised in the wild type, at 2 bp from the IES boundary. However, a more likely explanation is that it arises from a PCR artifact. Indeed, we have also observed a 2-bp deletion within a similar stretch of eight successive TAs (between nucleotides 1,938 and 1,953 of the wild-type sequence) in another

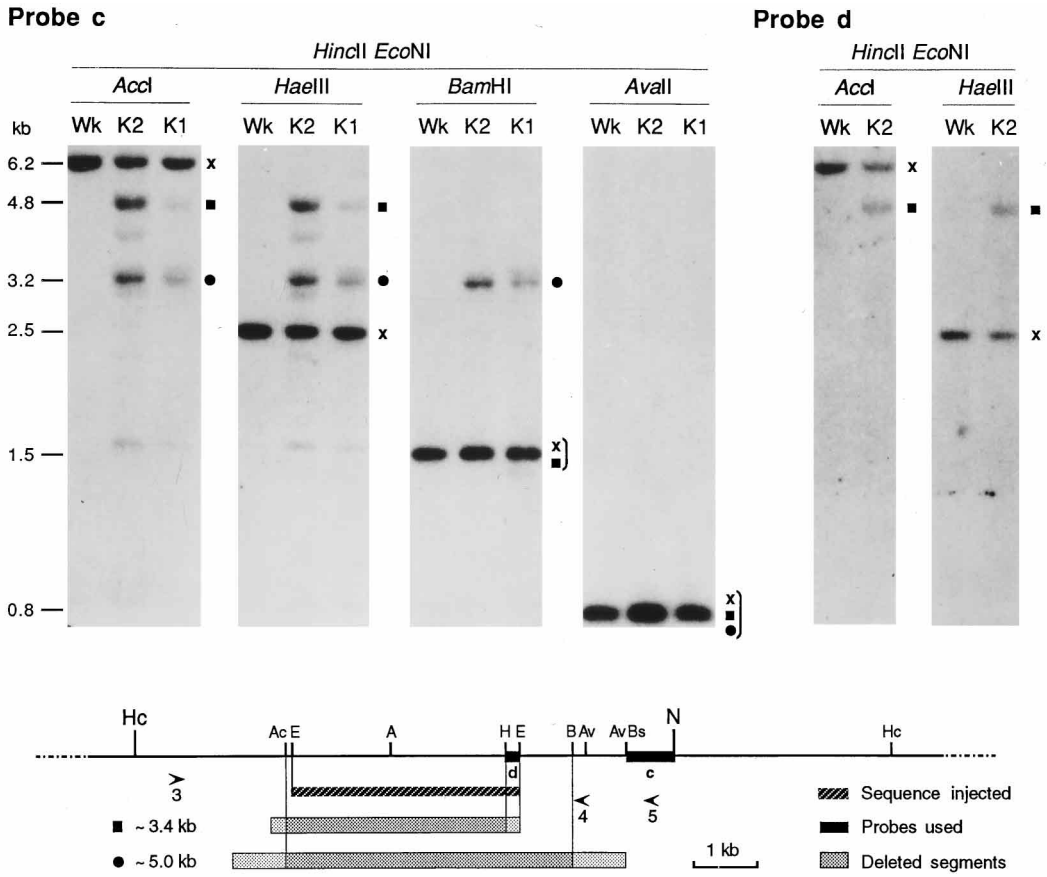

FIG. 4. Mapping of internal deletions of the LK1 sequence in clones K1 and K2. Genomic DNA from clones K1, K2, and Wk (a wild-type control) was digested with *Hinc*II, *Eco*NI, and an additional enzyme, as indicated above each panel. Probes c and d (see map) reveal subfragments of the *Hinc*II-*Eco*NI segment. The wild-type macronuclear version is indicated by an x; the two main deleted forms, corresponding to the 3.4- and 5.0-kb deletions, are indicated by a square and a circle, respectively. Mapped deletions are shown as shaded boxes below the wild-type restriction map; lighter-shaded ends indicate the uncertainty in the positions of deletion boundaries. The arrowheads indicate the positions and orientations of PCR primers 3 to 5. Restriction sites: A, *Asp*718; Ac, *Acc*I; Av, *Ava*II; B, *Bam*HI; Bs, *Bst*NI; E, *Eco*RI; H, *Hae*III; Hc, *Hinc*II; N, *Eco*NI.

cloned PCR product (deletion 11), about 100 bp away from the main deletion junction. PCR amplification of stretches of repeated dinucleotides has been shown to produce 2-bp deletions, probably through polymerase slippage (33); both deletions and insertions could result from the looping out of one strand during replication. If PCR slippage had increased the number of TAs of junction 1 from six to eight, then the original deletion would have occurred between 5′-TA-3′ direct repeats. Although the occurrence of PCR slippage could cast doubts on the structure of junctions containing long tracts of TAs (deletions 7 to 10), it does not call into question the identification of deletion boundaries; the absence of deleted products in the amplification of wild-type macronuclear DNA shows that large deletions between distant TA tracts are not attributable to a PCR artifact.

DISCUSSION

Induction of macronuclear deletions by plasmid transformation is a general and sequence-specific process. Previous work has shown that the induction of *G* gene deletions in the new macronucleus by transformation with plasmid pXΔ4 is sequence specific: a plasmid containing a totally unrelated sequence has no effect on the processing and amplification of the *G* gene. The present work shows that three additional plasmids containing different segments of the *G* gene region can induce macronuclear deletions in this genomic region even though some of them contain no common sequence. Furthermore, the induction of internal deletions of the LK1 sequence by transformation with pLK1 indicates that the phenomenon is not limited to surface antigen genes or subtelomeric regions of the macronuclear genome.

One important implication of LK1 deletions is that the effect does not necessarily entail chromosome breakage and telomere formation; as in the programmed elimination of IESs, it can lead to the rejoining of flanking sequences. Terminal deletions induced by *G* gene plasmids include the whole segment between the injected sequence and the wild-type macronuclear telomere (16 kb in the case of EBG1), whereas pLK1, which is located 72 kb upstream of the same telomere, induces internal deletions only slightly larger than itself. The idea that terminal deletions result from the proximity of the injected sequence to a chromosome fragmentation event is supported by additional experiments, presented elsewhere (6), showing that two plasmids from different genomic regions can cause either terminal or internal deletions. These sequences are located near facultative chromosome breakage sites and thus are found in the wild-type macronucleus in both subtelomeric and internal positions. Transformation of the maternal macronucleus affects both types of chromosomes in sexual progeny, resulting in

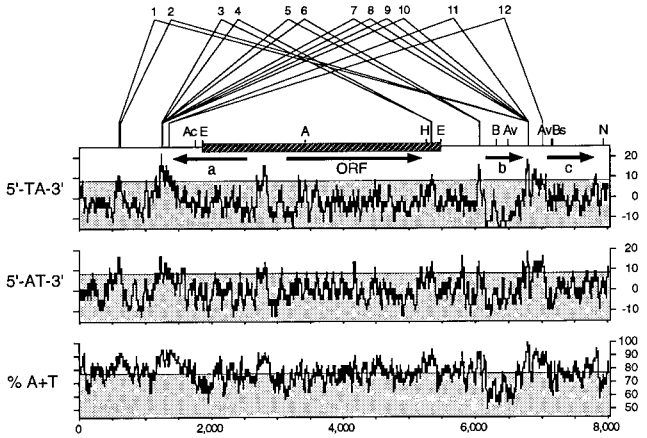

FIG. 5. Sequence analysis. Boundaries of pLK1-induced deletions are positioned on the map of the wild-type macronuclear sequence for each of the 12 sequenced junctions. The LK1 sequence is shown as a hatched box. Arrows indicate the positions and orientations of probable protein-coding genes (see the text). The top graphs represent the local frequencies of TA and AT dinucleotides (in a 40-bp window moved along the sequence by 1-bp increments), expressed as the percentage observed minus the percentage expected from the base composition of the whole sequence. The area of the graph below 8% of overrepresentation is shaded to highlight peaks. The bottom graph represents the local percentage of A and T nucleotides (in the same 40-bp window). The area of the graph below the mean value (77%) is shaded. Abbreviations: A, *Asp*718; Ac, *Acc*I; Av, *Ava*II; B, *Bam*HI; Bs, *Bst*NI; E, *Eco*RI; H, *Hae*III; N, *Eco*NI; ORF, open reading frame.

terminal deletions when the chromosome is fragmented nearby and in internal deletions when it is not.

Taken together, these results suggest that the primary effect of plasmid transformation is to decrease the copy number of homologous sequences in the new macronucleus rather than to induce specific rearrangements; the boundaries of underrepresented segments will necessarily show some kind of rearrangement. It is not known at what stage of macronuclear development and genome amplification the targeted sequences are deleted or, indeed, whether they are deleted at all or simply not replicated. However, the multiplicity of deletion end points observed in single macronuclei suggests that the process operates only after a substantial amplification of the diploid genome. Deletions were induced by all seven plasmids tested, in four different regions; the phenomenon therefore must be quite general. Its sequence specificity, evident from the fact that injected plasmids only affect rearrangements in the homologous genomic region, can also be seen within one genomic region, as plasmids containing nonoverlapping segments of the *G* gene region induce different terminal deletions.

The exact positions of deletion boundaries are determined by the germ line sequence. The mapping of *G* gene terminal deletions (Fig. 1) shows that in all cases a fraction of deletion breakpoints are located upstream of the injected sequence. However, the overlap between the mutant telomere addition region and the injected sequence is not the same in all cases; in pEG1-induced deletions, the telomere addition region is entirely located upstream of sequence EG1. This observation rules out an earlier hypothesis (29) according to which mutant telomere positions could arise in the new macronucleus through a mechanism copying the telomeres added onto plasmid sequences in the maternal macronucleus. Moreover, the fact that the telomere addition region induced by pEG1 is the same as that induced by pXΔ4 (which maps at the 5′ end of the *G* gene coding sequence) suggests that rearrangements preferentially occur in specific regions of the germ line sequence.

Similar conclusions can be drawn from the analysis of pLK1-induced internal deletions. Indeed, the same set of alternative deletion boundaries (mostly located outside the LK1 sequence) is observed in two independent postautogamous clones, indicating that their positions are determined by local particularities of the germ line sequence. The sequencing of 12 deletion junctions identified a number of deletion hot spots, each showing limited microheterogeneity in the precise recombination site. The two most frequently used hot spots in this sample (left boundaries of deletions 3 to 10 and right boundaries of deletions 1, 2, and 7 to 11) contain eight and five consecutive

FIG. 6. The 12 sequenced junctions of pLK1-induced internal deletions (del.). Junctions 2 to 12 appear to have been produced by recombination between two short direct repeats (shaded boxes), one of which was left in the rearranged macronuclear molecule. The number above each box refers to the first nucleotide of the repeat. The deleted sequence is shown in lowercase letters. The extra TA of junction 1 is shown in boldface letters.

repeats of the dinucleotide 5′-TA-3′, respectively. This prompted us to examine whether the locations of hot spots could be determined by specific sequence features. The top graph in Fig. 5 represents the local richness in 5′-TA-3′ dinucleotides, computed in a 40-bp window sliding along the whole sequence. Quite strikingly, sequenced deletion boundaries coincide with the highest peaks in TA density. For comparison, Fig. 5 also shows the density profile of 5′-AT-3′ dinucleotides and the local percentage of A and T nucleotides. Not surprisingly, the three graphs are correlated; however, the TA graph appears to have the highest predictive value. Thus, the mechanism by which possible boundaries for the deletion of the targeted sequence are selected may involve direct recognition of TA-rich sequences or use some other, strongly correlated criterion. Figure 5 shows that peaks in TA density also neatly delineate putative protein-coding sequences, so that genes appear to behave as the smallest deletion units. A similar mechanism may select the boundaries of terminal deletions, as transformation with a fragment internal to the *G* gene coding sequence (EG1) results in the deletion of the whole gene.

Relation of plasmid-induced deletions to IES excision. The sequence analysis indicates that LK1 internal deletions occur between two short direct repeats of alternating T's and A's, raising the possibility that the mechanism of plasmid-induced deletions, like IES excision, invariably involves recombination between two TA dinucleotides (22). Because of these direct repeats, it is impossible to pinpoint the exact sites of recombination in each case. Nevertheless, it is clear that several different sites can be used within a single hot spot, as the direct repeats of different junctions are often not overlapping (see, for example, the left direct repeats of deletions 9 and 10 or the right direct repeats of deletions 3 and 4, 5 and 6, or 2 and 11). A more limited microheterogeneity of deletion end points has also been reported for some *Paramecium* IESs located in noncoding sequences (13). Assuming that any one of the TAs in the direct repeats could have been used for recombination, we looked for adjacent sequences fitting the poorly conserved, degenerate consensus of IES ends, 5′-TAYAGYNR-3′, which was identified through a statistical analysis of sequences internal to the TA boundaries of IESs (22). A good fit is found in only one case: at the right boundary of deletion 5, the sequence between nucleotides 6,071 and 6,064 reads 5′-TATAGTAG-3′ going into the deleted segment. Even the most significant position of the consensus sequence (the G in position 5) is clearly lacking at all other deletion ends. Thus, while the two deletion processes may use the same basic recombination mechanism, the greater heterogeneity of plasmid-induced deletion junctions could be linked to the absence of consensus.

It should be noted that in both plasmid-induced deletions and IES excision, the mechanism determining whether the sequence should be deleted or amplified may be independent from the definition of deletion end points. The excision of some IESs was shown to be under an epigenetic, sequence-specific control exerted by the maternal macronucleus (13a, 14); an exciting possibility is that the epigenetic programming of deletions by transformation of the maternal macronucleus draws on the same mechanism.

Nucleic acids as *trans*-acting factors. The experiments presented here reveal the existence of *trans*-acting factors that modulate the amplification and rearrangement of specific regions of the germ line genome. The molecular nature of these factors is unknown; sequence specificity could be explained either by the production of sequence-specific protein factors from plasmids replicating in the old macronucleus (or being titrated by them) or by direct recognition of germ line sequences by homologous nucleic acid sequences originating from the old macronucleus. We cannot envision how each of the plasmids tested could encode a protein factor specifically affecting the developmental processing of its genomic region of origin. It is also very difficult to believe that each of these plasmids could contain a binding site for a specific protein factor that is required for normal processing of the homologous germ line sequence so that titration of the factor by the old macronucleus would prevent its action on developing macronuclei. Indeed, tested plasmids were randomly chosen, suggesting that a large fraction of arbitrarily defined segments of the macronuclear genome are able to induce specific deletions; under this assumption, the huge number of different protein factors required to process the whole genome would take up most of its own coding capacity. The sequence specificity of the effect is more easily explained by the hypothesis that the *trans*-acting factors are nucleic acids originating from the old macronucleus and that they act by pairing with germ line sequences during macronuclear development. However, genetic material from the old macronucleus is not amplified to become part of the new macronuclear genome; no recognizable plasmid sequence (bacterial sequence or different allele) can be detected in the macronucleus of sexual progeny of transformed clones by Southern blotting. The maternal *trans*-acting factors could be RNA molecules, or they could be DNA fragments that influence the processing of the germ line genome but are not themselves amplified.

One difficult requirement of this hypothesis is that nucleic acids have to be transferred between different nuclei in the same cytoplasm. While there is no direct evidence for such a transfer, it has already been postulated to account for several sequence-specific maternal effects on developmental rearrangements of the zygotic genome (for reviews, see references 30 and 31). A model effect of the maternal macronuclear genome on the positioning of macronuclear telomeres in the *G* gene region has led to the suggestion that the *trans*-acting factors carry sequence information (29). A mechanism involving pairing has also been proposed to account for the ability of several nonoverlapping DNA segments from the *A* gene, after injection into the d48 maternal macronucleus, to prevent *A* gene terminal deletion of the developing macronucleus, thus permanently rescuing the d48 macronuclear mutation in sexual progeny (21, 48). The experiment has been repeated with a macronuclear deletion of the *B* gene, a related surface-antigen gene; despite the fact that the rescuing ability is not confined to a particular sequence within the *A* gene, a study of cell lines carrying both *A* gene and *B* gene macronuclear deletions has shown that the rescue mechanism is gene specific (41), which supports the pairing hypothesis. Finally, a formally similar *trans*-nuclear effect, the inhibition of excision of some IESs by homologous sequences present in the maternal macronucleus, may also involve pairing (13a, 14).

While all of these results point to the same general conclusion, that transformation of the old macronucleus with cloned macronuclear segments specifically affects the processing of the homologous germ line sequences, it is unclear why it appears to induce macronuclear deletions in some cases and prevent them in others. One difference between the two types of experiments is that in rescue experiments, the injected fragments are already missing from the recipient macronucleus. We therefore tested the effect of injection of plasmids pEBG1 and pEG1 into the macronucleus of a pEBG1-induced mutant. Reversion to wild-type processing of the *G* gene was not enhanced in the following generation compared to uninjected controls. For each plasmid, the same terminal deletion is induced after injection into wild-type or mutant macronuclei.

It is unlikely that the discrepancy arises from the use of two different sibling species, *P. primaurelia* in our study and *P. tetraurelia* in the rescue experiments, because transformation of the maternal macronucleus can also induce underrepresentation of homologous sequences in *P. tetraurelia* (14). Rather, the difference may be attributable to technical procedures for transformation. It was shown that deletion induction is dependent on the presence of a high copy number of the transforming plasmid (14, 29). Furthermore, the process is much less efficient (relative to plasmid copy number) when *G* gene plasmids are injected as linear molecules (data not shown). One difference between the injection of circular and linear molecules is that circular molecules, following linearization in the macronucleus, have telomeric repeats added at many different points of the sequence. We have also shown that most, if not all, of the multimers obtained from circular molecules formally correspond to nonhomologous recombination (unpublished results); they are presumably produced by end-to-end joining of many different types of linearized monomers before telomere addition. These multimers therefore present a wide variety of sequence rearrangements disrupting homology with the germ line sequence, whereas multimers obtained from linear molecules correspond to end-to-end joining of monomers that have all been cut at the same site and contain only a limited number of sequence discontinuities.

The pairing of plasmid sequences containing many homology-disrupting discontinuities may thus be responsible for the deletion of germ line sequences. This idea is reminiscent of a model developed to explain repeat-induced gene silencing in *Arabidopsis thaliana*, in which it was proposed that the pairing of sequences containing homologous segments flanked by heterologous sequences induces condensation of the chromatin into a nontranscribable state (2, 46). Three-way structures formed at the homology/heterology boundaries may be recognized by specific factors, leading to chromatin remodelling or DNA methylation (4). An epigenetic imprint imposed on specific sequences by pairing interactions could then be translated into the observed deletions; recent evidence indeed suggests that most of the germ line-specific DNA is eliminated as heterochromatin in the related ciliate *Tetrahymena thermophila* (26). Heterochromatin spreading could explain the deletion of sequences located between the injected sequence and nearby chromosome fragmentation regions in our experiments.

On the other hand, some germ line sequences may also contain *cis*-acting signals that spontaneously trigger heterochromatin formation during macronuclear development. In *Paramecium* surface-antigen genes, the presence of ~200-bp tandem repeats in the coding sequence may constitute such a signal, as repeated sequences favor heterochromatin formation in *Arabidopsis thaliana* (46) and *Drosophila melanogaster* (11, 36), and perhaps also in mice (10), probably as a consequence of intramolecular paired structures. Germ line sequences that can be amplified in the developing macronucleus only if they are already present in the maternal macronucleus, such as the *A* and *B* genes and some IESs, might require pairing with colinear maternal molecules to prevent spontaneous heterochromatin formation and deletion.

Further work is needed to clarify the role of maternal sequences in the epigenetic programming of *Paramecium* genome rearrangements. The system is unique in that it appears to involve a *trans*-nuclear comparison of the zygotic genome with the maternal somatic genome; nevertheless, the likely involvement of pairing-induced epigenetic modifications clearly relates it to a number of homology-dependent gene silencing phenomena in other eukaryotes (for reviews, see references 15, 27, 28, 32, and 35).

ACKNOWLEDGMENTS

This work was supported by grants 30 and 22/95 from the Groupement de Recherches et d'Etudes sur les Génomes, Gif-sur-Yvette, France, and by grant 1374 from the Association pour la Recherche sur le Cancer, Villejuif, France. K.D. and S.D. are recipients of doctoral fellowships from the Ministère de l'Enseignement Supérieur et de la Recherche and from the Association pour la Recherche sur le Cancer, respectively.

REFERENCES

1. **Amar, L.** 1994. Chromosome end formation and internal sequence elimination as alternative genomic rearrangements in the ciliate *Paramecium*. J. Mol. Biol. **236:**421–426.
2. **Assaad, F. F., K. L. Tucker, and E. R. Signer.** 1993. Epigenetic repeat-induced gene silencing (RIGS) in *Arabidopsis*. Plant Mol. Biol. **22:**1067–1085.
3. **Baroin, A., A. Prat, and F. Caron.** 1987. Telomeric site position heterogeneity in macronuclear DNA of *Paramecium primaurelia*. Nucleic Acids Res. **15:**1717–1728.
4. **Bestor, T. H., and B. Tycko.** 1996. Creation of genomic methylation patterns. Nat. Genet. **12:**363–367.
5. **Caron, F.** 1992. A high degree of macronuclear chromosome polymorphism is generated by variable DNA rearrangements in *Paramecium primaurelia* during macronuclear differentiation. J. Mol. Biol. **225:**661–678.
6. **Caron, F., A. Butler, A. Le Mouël, and E. Meyer.** Alternative deletions of germ-line sequences healed by religation or by telomere addition during macronuclear development in *Paramecium*. Submitted for publication.
7. **Chu, G., D. Vollrath, and R. W. Davis.** 1986. Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science **234:**1582–1585.
8. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA **81:**1991–1995.
9. **Coyne, R. S., D. L. Chalker, and M.-C. Yao.** 1996. Genome downsizing during ciliate development: nuclear division of labor through chromosome restructuring. Annu. Rev. Genet. **30:**557–578.
10. **Dobie, K., M. Mehtali, M. McClenaghan, and R. Lathe.** 1997. Variegated gene expression in mice. Trends Genet. **13:**127–130.
11. **Dorer, D. R., and S. Henikoff.** 1994. Expansions of transgene repeats cause heterochromatin formation and gene silencing in *Drosophila*. Cell **77:**993–1002.
12. **Dryl, S.** 1959. Antigenic transformation in *Paramecium aurelia* after homologous antiserum treatment during autogamy and conjugation. J. Protozool. **6**(Suppl.)**:**25.
13. **Dubrana, K., A. Le Mouël, and L. Amar.** Deletion endpoint allele specificity in the developmentally regulated elimination of an internal sequence (IES) in *Paramecium*. Nucleic Acids Res., in press.

13a. **Duharcourt, S.** Unpublished data.

14. **Duharcourt, S., A. Butler, and E. Meyer.** 1995. Epigenetic self-regulation of the excision of an internal eliminated sequence in *Paramecium tetraurelia*. Genes Dev. **9:**2065–2077.
15. **Flavell, R. B.** 1994. Inactivation of gene expression in plants as a consequence of specific sequence duplication. Proc. Natl. Acad. Sci. USA **91:**3490–3496.
16. **Forney, J. D., and E. H. Blackburn.** 1988. Developmentally controlled telomere addition in wild-type and mutant paramecia. Mol. Cell. Biol. **8:**251–258.
17. **Gilley, D., J. R. Preer, Jr., K. J. Aufderheide, and B. Polisky.** 1988. Autonomous replication and addition of telomerelike sequences to DNA microinjected into *Paramecium tetraurelia* macronuclei. Mol. Cell. Biol. **8:**4765–4772.
18. **Godiska, R. D., K. J. Aufderheide, D. Gilley, P. Hendrie, T. Fitzwater, L. B. Preer, B. Polisky, and J. R. Preer, Jr.** 1987. Transformation of *Paramecium* by microinjection of a cloned serotype gene. Proc. Natl. Acad. Sci. USA **84:**7590–7594.
19. **Jessop-Murray, H., L. D. Martin, D. Gilley, J. R. Preer, Jr., and B. Polisky.** 1991. Permanent rescue of a non-Mendelian mutation of *Paramecium* by microinjection of specific DNA sequences. Genetics **129:**727–734.
20. **Kim, C. S., J. R. Preer, Jr., and B. Polisky.** 1992. Bacteriophage lambda fragments replicate in the *Paramecium* macronucleus: absence of active copy number control. Dev. Genet. **13:**97–102.
21. **Kim, C. S., J. R. Preer, Jr., and B. Polisky.** 1994. Identification of DNA segments capable of rescuing a non-Mendelian mutant in *Paramecium*. Genetics **136:**1325–1328.
22. **Klobutcher, L. A., and G. Herrick.** 1995. Consensus inverted terminal repeat sequence of *Paramecium* IESs: resemblance to termini of Tc1-related and *Euplotes* Tec transposons. Nucleic Acids Res. **23:**2006–2013.
23. **Klobutcher, L. A., and G. Herrick.** 1997. Developmental genome reorganization in ciliated protozoa: the transposon link. Prog. Nucleic Acids Res. Mol. Biol. **56:**1–62.
24. **Kobayashi, S., and S. Koizumi.** 1990. Characterization of non-Mendelian

and Mendelian mutant strains by micronuclear transplantation in *Paramecium tetraurelia*. J. Protozool. **37:**489–492.
25. **Koizumi, S., and S. Kobayashi.** 1989. Microinjection of plasmid DNA encoding the A surface antigen of *Paramecium tetraurelia* restores the ability to regenerate a wild-type macronucleus. Mol. Cell. Biol. **9:**4398–4401.
26. **Madireddi, M. T., R. S. Coyne, J. F. Smothers, K. M. Mickey, M.-C. Yao, and D. Allis.** 1996. Pdd1p, a novel chromodomain-containing protein, links heterochromatin assembly and DNA elimination in *Tetrahymena*. Cell **87:**75–84.
27. **Matzke, M. A., and A. J. M. Matzke.** 1995. Homology-dependent gene silencing in transgenic plants: what does it really tell us? Trends Genet. **11:**1–3.
28. **Matzke, M. A., A. J. M. Matzke, and W. B. Eggleston.** 1996. Paramutation and transgene silencing: a common response to invasive DNA? Trends Plant Sci. **1:**382–388.
29. **Meyer, E.** 1992. Induction of specific macronuclear developmental mutations by microinjection of a cloned telomeric gene in *Paramecium primaurelia*. Genes Dev. **6:**211–222.
30. **Meyer, E., and S. Duharcourt.** 1996. Epigenetic programming of developmental genome rearrangements in ciliates. Cell **87:**9–12.
31. **Meyer, E., and S. Duharcourt.** 1996. Epigenetic regulation of programmed genomic rearrangements in *Paramecium aurelia*. J. Eukaryot. Microbiol. **43:**453–461.
32. **Meyer, P.** 1996. Homology-dependent gene silencing in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47:**23–48.
33. **Murray, V., C. Monchawin, and P. R. England.** 1993. The determination of the sequences present in the shadow bands of a dinucleotide repeat PCR. Nucleic Acids Res. **21:**2395–2398.
34. **Prescott, D. M.** 1994. The DNA of ciliated protozoa. Microbiol. Rev. **58:** 233–267.
35. **Rossignol, J.-L., and G. Faugeron.** 1994. Gene inactivation triggered by recognition between DNA repeats. Experientia **50:**307–317.
36. **Sabl, J. F., and S. Henikoff.** 1996. Copy number and orientation determine the susceptibility of a gene to silencing by nearby heterochromatin in *Drosophila*. Genetics **142:**447–458.
37. **Sambrook, J., E. F. Fritch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
38. **Saveliev, S. V., and M. M. Cox.** 1994. The fate of deleted DNA produced during programmed genomic deletion events in *Tetrahymena thermophila*. Nucleic Acids Res. **22:**5695–5701.
39. **Scott, J., C. Leeck, and J. Forney.** 1993. Molecular and genetic analysis of the B type surface protein gene from *Paramecium tetraurelia*. Genetics **133:**189–198.
40. **Scott, J., C. Leeck, and J. Forney.** 1994. Analysis of the micronuclear B type surface protein gene in *Paramecium tetraurelia*. Nucleic Acids Res. **22:**5079–5084.
41. **Scott, J. M., K. Mikami, C. L. Leeck, and J. D. Forney.** 1994. Non-Mendelian inheritance of macronuclear mutations is gene specific in *Paramecium tetraurelia*. Mol. Cell. Biol. **14:**2479–2484.
42. **Sonneborn, T. M.** 1970. Methods in *Paramecium* research. Methods Cell Physiol. **4:**241–339.
43. **Sonneborn, T. M.** 1974. *Paramecium aurelia*, p. 469–594. *In* R. C. King (ed.), Handbook of genetics: plants, plant viruses and protists, vol. 2. Plenum Press, New York, N.Y.
44. **Steele, C. J., G. A. Barkocy-Gallagher, L. B. Preer, and J. R. Preer, Jr.** 1994. Developmentally excised sequences in micronuclear DNA of *Paramecium*. Proc. Natl. Acad. Sci. USA **91:**2255–2259.
45. **Yao, M.-C., and C.-H. Yao.** 1994. Detection of circular excised DNA deletion elements in *Tetrahymena thermophila* during development. Nucleic Acids Res. **22:**5702–5708.
46. **Ye, F., and E. R. Signer.** 1996. RIGS (repeat-induced gene silencing) in *Arabidopsis* is transcriptional and alters chromatin configuration. Proc. Natl. Acad. Sci. USA **93:**10881–10886.
47. **You, Y., K. Aufderheide, J. Morand, K. Rodkey, and J. Forney.** 1991. Macronuclear transformation with specific DNA fragments controls the content of the new macronuclear genome in *Paramecium tetraurelia*. Mol. Cell. Biol. **11:**1133–1137.
48. **You, Y., J. Scott, and J. Forney.** 1994. The role of macronuclear DNA sequences in the permanent rescue of a non-Mendelian mutation in *Paramecium tetraurelia*. Genetics **136:**1319–1324.